International Journal of Pharmaceutics 476 (2014) 108–115

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Increasing the stability of curcumin in serum with liposomes or hybrid drug-in-cyclodextrin-in-liposome systems: A comparative study

Ahmed H. Matloob [a], Spyridon Mourtas [a], Pavlos Klepetsanis [a,b], Sophia G. Antimisiaris [a,b,*]

[a] Laboratory of Pharmaceutical Technology, Department of Pharmacy, University of Patras, Rio 26510, Greece
[b] Foundation for Research and Technology Hellas, Institute of Chemical Engineering Sciences, FORTH/ICES, Rio 26504, Greece

ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form 15 September 2014
Accepted 26 September 2014
Available online 28 September 2014

Keywords:
Curcumin
Liposome
Cyclodextrin
Inclusion complex
Stability
Serum

Pubchem classification:
Curcumin (CID: 969516)
Hydroxyl-propyl-beta-cyclodextrin
?(CID: 44134771)
Hydroxyl-propyl-gamma-cyclodextrin
(CID: 119174)

ABSTRACT

Curcumin (CURC) was incorporated in liposomes as free drug or after formation of hydropropyl-β- or hydroxypropyl-γ-cyclodextrin (HPβCD or HPγCD) complexes prepared by coprecipitation and characterized by X-ray diffractometry. Liposomes encapsulating CURC as free drug or CD-complexes (hybrid formulations) were prepared by the dehydration–rehydration vesicle (DRV) method followed by extrusion, and characterized for size, zeta-potential and CURC loading. CURC stability (at 0.01 and 0.05 mg/mL) in 80% (v/v) fetal bovine serum (FBS) was evaluated at 37 °C. Results demonstrate that HPβCD stabilizes CURC more than HPγCD, but liposome encapsulation provides substantially more protection, than CDs. CURC stabilization is similar, when encapsulated as free compound or CD-complex. However, the last method increases CURC loading by 23 times (depending on the lipid composition of liposomes and the CD used), resulting in higher solubility. The stability profile of CURC in serum depends on the composition of liposomes and CURC concentration, since at lower concentrations larger CURC fractions are protected due to protein binding. Compared to the corresponding CD complexes, hybrid formulations provide intermediate CURC solubility (comparable to HPβCD) but profoundly higher stabilization.

1. Introduction

Curcumin (CURC), a hydrophobic polyphenol is the principal component of turmeric and has been shown to have antioxidant, anti-inflammatory and anticancer properties, in addition to other interesting biological activities (Anand et al., 2008). Due to its pharmacological efficacy, CURC has been intensively investigated for a wide range of applications (Epstein et al., 2010) however, a major problem to use CURC as a therapeutic agent is its poor bioavailability (Anand et al., 2007). CURC bioavailability is related to its hydrolytic instability and low aqueous solubility at physiological pH. Indeed, a very low aqueous solubility of 11 ng/mL, was reported for curcumin, (Tønnesen et al., 2002).

Methods to increase CURC solubility and/or protect CURC from hydrolytic degradation, as complexation using cyclodextrins (CDs) or encapsulation within nanoparticulate carriers, have been extensively investigated (Basnet et al., 2012; Baglole et al. 2005; Sahu et al., 2011; Seo et al., 2012; Singh et al., 2012; Tang et al., 2002; Tomren et al., 2007; Tønnesen, 2006; Wan et al., 2012; Zhao et al., 2012).

A number of molecules are solubilized in CD solutions through formation of inclusion complexes (Loftsson and Duchêne, 2007); while CD inclusion-complex formation is known to modulate the chemical stability of drug molecules, usually (but not always) leading to retardation of degradation (Loftsson, 1995; Loftsson and Brewster, 1996). Several types of CD derivatives are available with different properties (size, shape or charge of the CD cavity), which influence the association constant (Kc) and/or the degradation rates of drugs which have formed inclusion complexes (Masson et al., 1998). CURC solubility in phosphate buffered saline (PBS) is approx. 20 μg/mL, and increases by many times (up to 10^4 times increases have been reported) when increasing quantities of different β-CD molecules are added in the solution (Tønnesen et al., 2002; Tomren et al., 2007; Yallapu et al., 2010).

Another well-known method to increase solubility of lipophilic drugs is by incorporation in liposomal membranes

* Corresponding author at: University of Patras, Department of Pharmacy, University Campus-Pharmacy, 26510 Rio-Patras, Greece. Tel.: +30 2610962332.
E-mail addresses: santimis@upatras.gr, santimis@hotmail.com (S.G. Antimisiaris).

http://dx.doi.org/10.1016/j.ijpharm.2014.09.041
0378-5173/

(Antimisiaris et al., 2007). Lipophilic drugs can distribute at high percentages in lipid bilayers, increasing their solubility in aqueous media (Nii and Ishii, 2005) in the form of aqueous liposomal dispersions. In the case of CURC, such incorporation in liposomal membranes may also increase its stability (and consequently its circulation period) in the blood, since CURC residence within liposomal membranes may protect it against hydrolytic degradation. However, when liposomes are administered in vivo, due to the high dilution factor in the blood (since mean blood volume is between 3 and 4 L), any lipophilic compounds incorporated within the liposome membranes will most possibly leak out from the membranes, at a degree which depends on the log*P* value of the particular compound, its aqueous solubility at physiological pH, and its affinity to plasma proteins. With the aim to avoid such high degrees of lipophilic-compound leakage from liposomal formulations upon injection in the blood, the formation of hybrid systems (**H**) to entrap lipophilic compounds in the aqueous compartment of liposomes (in the form of aqueous soluble CD-complexes), was proposed (McCormack and Gregoriadis, 1994; Fatouros et al., 2001). Several studies have been carried out in order to determine the potential interactions which take place in such complex systems (Fatouros et al., 2001; Hatzi et al., 2007; Joguparthi and Anderson, 2008; Piel et al., 2007; Chen et al., 2014), as those between CD molecules and constituents of the liposome membrane (cholesterol and phospholipids), in order to select the best possible combination of ingredients for stable formulations. In fact, it was demonstrated that each drug molecule case has to be investigated individually, since the association constant with the different CD molecules differ, having a high impact on the stability of the hybrid (drug-in-CD-in-liposome) system (Hatzi et al., 2007).

Herein, we investigate the potential of formulating such hybrid CURC-in-CD-in-liposome systems with the aim to increase the solubility and stability of CURC in blood. The current study is novel, since although (as also mentioned above), several attempts have been made to increase the solubility and/or stability of CURC, by CD-complex formation (Baglole et al., 2005; Tomren et al., 2007; Tang et al., 2002; Tønnesen, 2006), and some by CURC incorporation in liposome (Chen et al., 2009), a very limited number of studies report on CURC-in-CD-in-liposome hybrid formulations (Agashe et al., 2011a,b,b; Dhule et al., 2012; Rahman et al., 2012). Furthermore, all of the previous studies primarily focus on the activity (in vitro and/or in vivo) of CURC when formulated in such systems, while no study to our best knowledge evaluated the potential of such complex formulations to protect CURC from degradation. Additionally, since it is known that serum proteins may protect CURC from hydrolytic degradation in plasma (Leung and Kee, 2009), and we expect that such complex hybrid formulations will have different CURC stabilization potential in absence and in presence of serum proteins, we selected to evaluate CURC stability in presence of such proteins, as a better in vitro model for predictions of the in vivo stability and biovailability of CURC.

Herein, two different CD molecules HP-β and HP-γ which have high aqueous solubility's and are known to form inclusion complexes with CURC (Tomren et al., 2007), were used. After formation of the CD CURC complexes, CURC-in-CD-in-liposome systems were prepared, and the stability of the hybrid formulations was evaluated in Fetal Bovine Serum (FBS), at two different CURC concentrations. Liposomes with different lipid compositions were evaluated; while conventional liposomes CURC-liposomes (in which CURC is incorporated in their lipid membrane), with the same lipid compositions with those of the hybrid systems, were also prepared and studied under identical experimental conditions, together with the CURCCD complexes. Thus by evaluating all three different formulation types, CURCCD complexes, CURC-Liposomes and hybrid CURC-in-CD-in-liposome systems, conclusions could be made about the CURC stabilization potential, of each specific component of the complex (or hybrid) system.

2. Materials and methods

2.1. Materials

Curcumin (>90%) and Cholesterol (99%) (Chol), were from SigmaAldrich. Phosphatidyl-choline (**PC**) and hydrogenated-PC (egg) (**HPC**) were purchased from Lipoid Gmbh, Germany. The chemical purity of the phospholipids was verified by TLC. 1,2-distearoyl-sn-glycerol-3-phosphoethanolamine-*N*-[methoxy (polyethyleneglycol)-2000] (PEG-lipid), was purchased from Avanti Polar Lipids. Hydroxypropyl-beta-cyclodextrin (HPβCD) was purchased from TCI Europe, and hydroxypropyl-gamma-CD (HPγCD), was from Aldrich. All solvents used were of analytical or HPLC grade and were purchased from Merck, (Germany). All other materials (as salts used for buffer preparation, reagents for lipid concentration determination and surfactants for liposome disruption) were of analytical grade and were from SigmaAldrich.

A Shimadzu UV-1205 spectrophotometer was utilized for measurement of liposomal lipid concentration, and a Shimadzu LC-20AB Prominence HPLC system (using a LiChrospher® 100, RP18 (5 μm) column) for measurement of CURC.

2.2. CyclodextrinCURC complex preparation and characterization

CDCURC complexes were prepared using the co-precipitation method (Tomren et al., 2007). Appropriate amounts of CURC and CD molecules (HPβCD or HPγCD) were dissolved in methanol and the solution was evaporated in a round bottomed flask, until the formation of a thin film which was subsequently scraped from the flask. The retrieved powder was dissolved in H_2O, to give a final CD concentration of 250 mg/mL. The CURC/CD complexes were purified by centrifugation, and characterized by XRD in order to verify the existence of the complex, since the ability of CURC to form complexes with the two CD molecules used herein, has been previously proven in many reports (Baglole et al., 2005; Tomren et al., 2007; Tang et al., 2002; Tønnesen, 2006).

2.3. Preparation of liposomes

For all liposome-types prepared, the lipid compositions used were: PC, PC/Chol (2:1 mol/mol), HPC, HPC/Chol (2:1 mol/mol) and HPC/Chol/PEG-lipid (2:1:0.08 mol/mol/mol). Conventional CURC-incorporating liposomes (**C**) were formulated by applying the lipid thin-film hydration technique followed by extrusion through polycarbonate filters for size reduction. In brief, multilamellar vesicles (MLV) were prepared by dissolving the appropriate weight of lipid (or lipids) and CURC (in a 10/1 lipid:CURC w/w ratio) with chloroform/methanol (2:1 v/v) in a round-bottomed-flask, and subsequently evaporating the solution under vacuum, until the formation of a thin lipid film, on the walls of the flask. The lipid film was hydrated with the appropriate volume of PBS buffer (pH 7.4) (usually 1 mL per 10 mg of lipid), at 25 °C in the case of PC liposomes and at 60 °C in the case of HPC. After complete lipid hydration and formation of liposomes, the vesicle dispersion was extruded through polycarbonate membranes with pore size of 400 nm, initially, and then 100 nm, using a Lipo-so-fast extruder (Avestin, Canada).

Hybrid (CURC-in-CD-in-liposome) formulations (**H**) were prepared according to the dehydrationrehydration vesicle (DRV) method, as previously reported (Fatouros et al., 2001; Antimisiaris, 2010). In brief, 1 mL of the CD/CURC complex (CD concentration